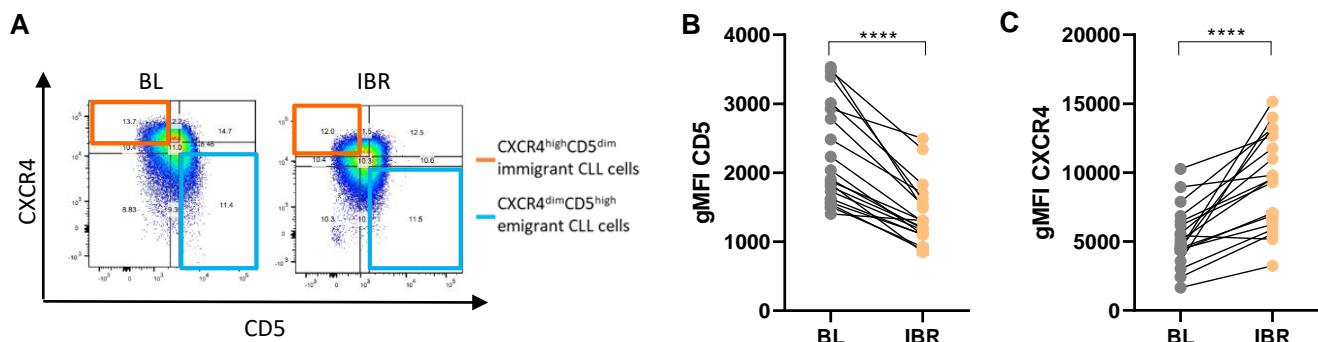


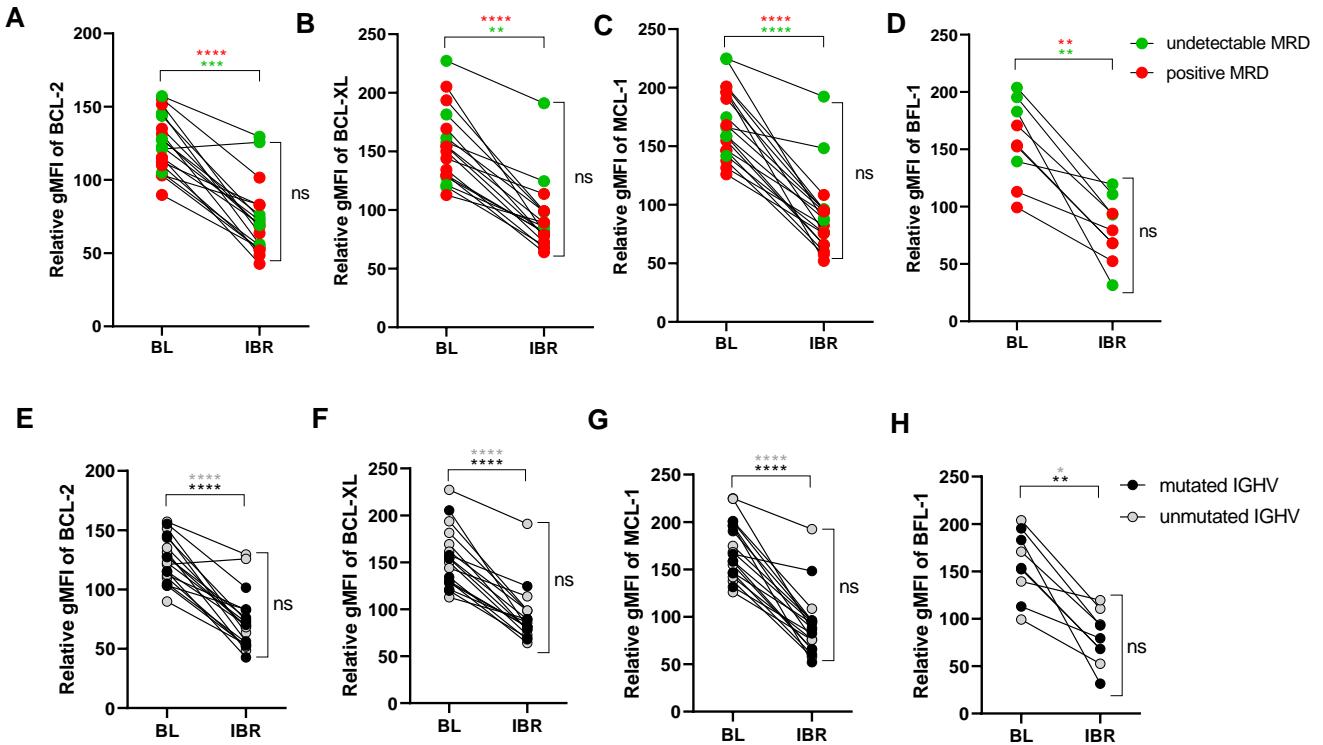
Supplemental Table 2. Reagents

Reagent	Supplier	Concentration
Ibrutinib for Hovon141 clinical trial	Janssen (Beerse, Belgium)	
Ibrutinib for in vitro experiments	Selleckchem (Texas, USA)	0.1 μ M
Venetoclax	Active Biochem (Bonn, Germany)	0.001-10 μ M
ODN2006 (Class B CpG oligonucleotide)	InvivoGen (Toulouse, France)	1 μ g/ml
goat F(ab) α -human IgM	Sanbio (Uden, The Netherlands)	20 μ g/ml
PAM3CSK4	InvivoGen (Toulouse, France)	10 μ g/ml
Poly-I:C	InvivoGen (Toulouse, France)	10 μ g/ml
LPS	Merck (Darmstadt, Germany)	1 μ g/ml
R837	InvivoGen (Toulouse, France)	5 μ g/ml
IFN- γ	Bio-Techne (Minneapolis, USA)	50 ng/ml
human IL-2	PeproTech (London, UK)	50 ng/ml
human IL-4	Thermo Fisher Scientific (Waltham, USA)	25 ng/ml
human IL-10	Bio-Techne (Minneapolis, USA)	50 ng/ml
human IL-15	PeproTech (London, UK)	50 ng/ml
human IL-21	Thermo Fisher Scientific (Waltham, USA)	50 ng/ml
human superkiller TRAIL	Enzo LifeSciences (Bruxelles, Belgium)	50 ng/ml
CXCL12	PeproTech (London, UK)	200 ng/ml
TNF α	R&D systems (Minneapolis, USA)	50 ng/ml



Supplemental Figure 1. Ibrutinib treatment changes CLL cells towards a LN immigrant phenotype

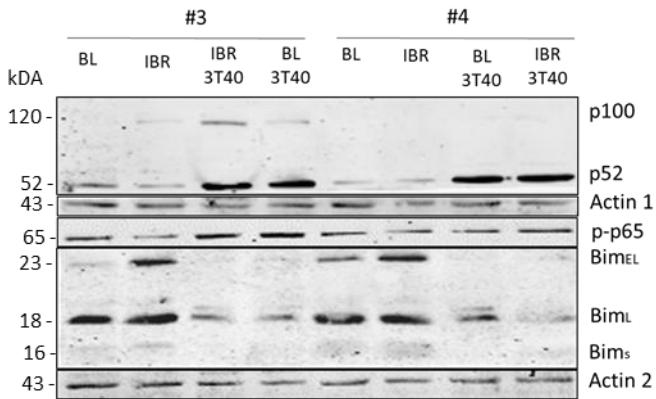
(A) Combined staining of CXCR4 and CD5 within the CLL population allows discrimination of LN emigrants (CD5^{high}CXCR4^{dim}) from LN immigrants (CXCR4^{high}CD5^{dim}) at baseline (BL) and after two months ibrutinib treatment (IBR). For all following experiments, an algorithm was applied to divide the total CLL population into 9 quadrants in an unbiased fashion. (B-C) Flow cytometry analysis of the expression levels of CD5 and the chemokine receptor CXCR4 (N=19) before and after ibrutinib treatment. TWO-Way Anova test was used for statistical analyses.



Supplemental Figure 2. Ibrutinib-mediated collapse of Bcl-2 family member expression does not correlate with mid-treatment MRD response with the combination

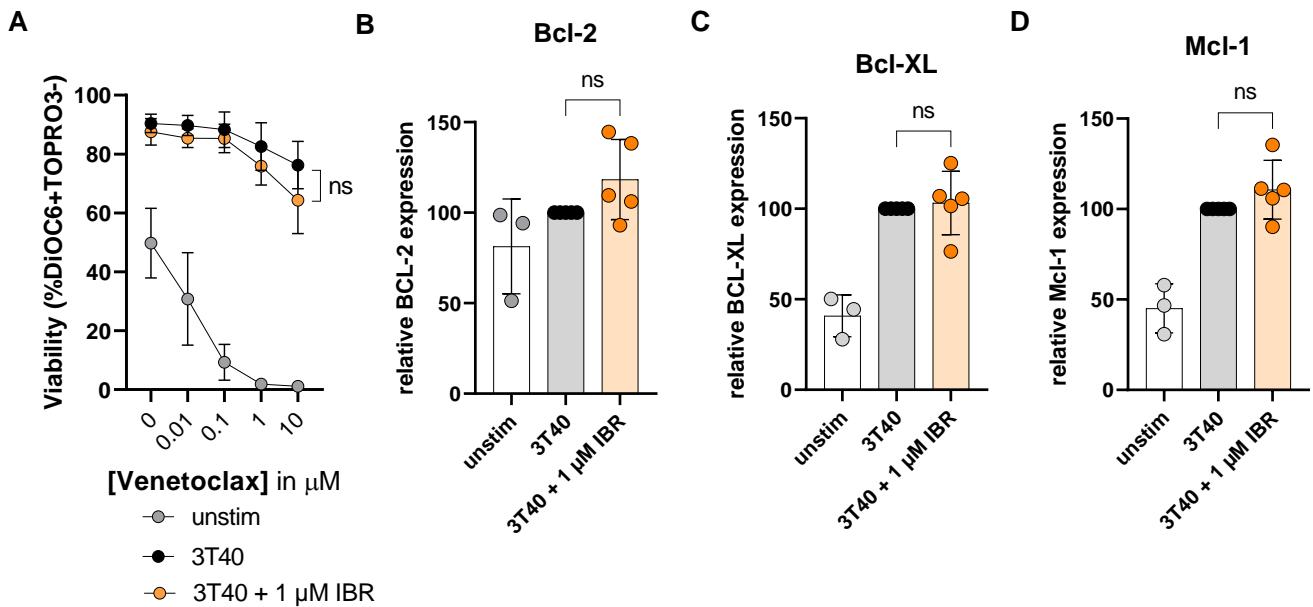
Immunological detection of Bcl-2 family members in LN emigrants before and after ibrutinib treatment (N=17; BFL-1 N=9). Data is divided based on positive MRD or undetectable MRD obtained at end cycle 9 of the trial (A-D) or IGHV mutational status (E-H), respectively.

gMFIs were normalized by setting the baseline LN immigrant population at 100%, and by subsequently plotting only the emigrant population. TWO-Way Anova test was used for statistical analyses.



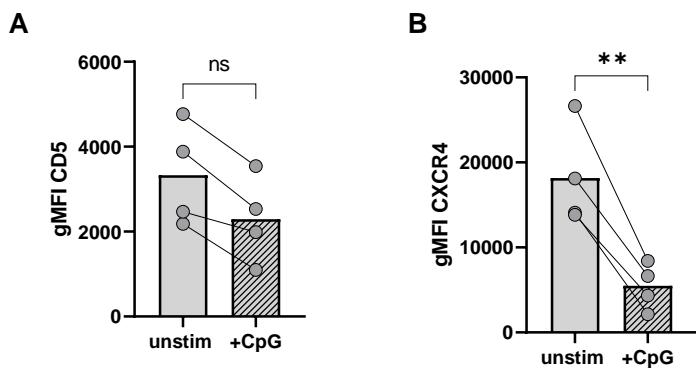
Supplemental Figure 3. Downstream mediators of CD40 are unaffected after ibrutinib treatment

(A) Western blot of peripheral blood collected from 2 patients. CLL cells obtained at baseline (BL) and after two months of ibrutinib treatment (IBR) were unstimulated or co-cultured on CD40L expressing fibroblasts (3T40) for 24h. Protein lysates were probed for NF- κ B proteins (p100, p52 and pp65), pro-apoptotic Bim and actin as loading control.

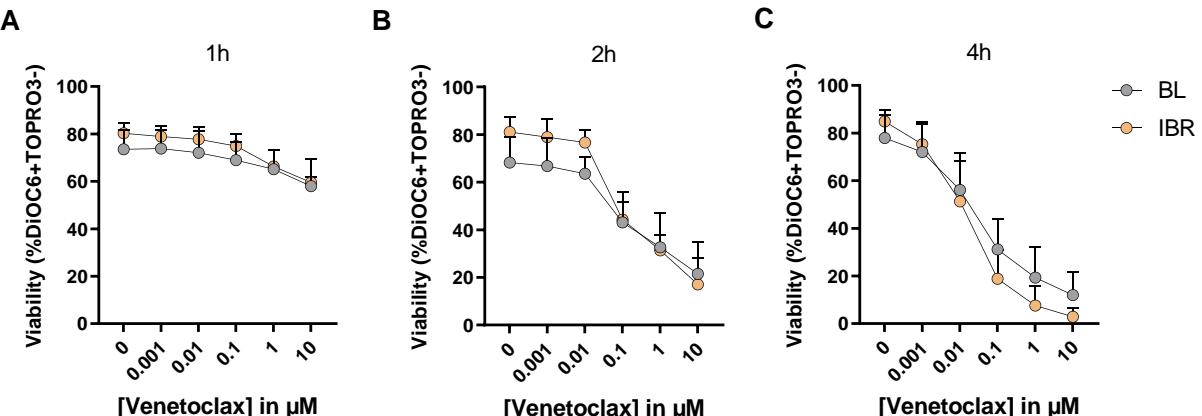


Supplemental Figure 4. *In vitro* experiments revealed no direct ibrutinib-mediated effects on CD40-induced venetoclax resistance

In vitro experiments using 1 μM ibrutinib for 24h showed no direct effects of ibrutinib on (A) venetoclax sensitivity and (B-D) Bcl-2 family proteins (N=5) as seen *in vivo*. Paired sample t-test was used for statistical analyses.

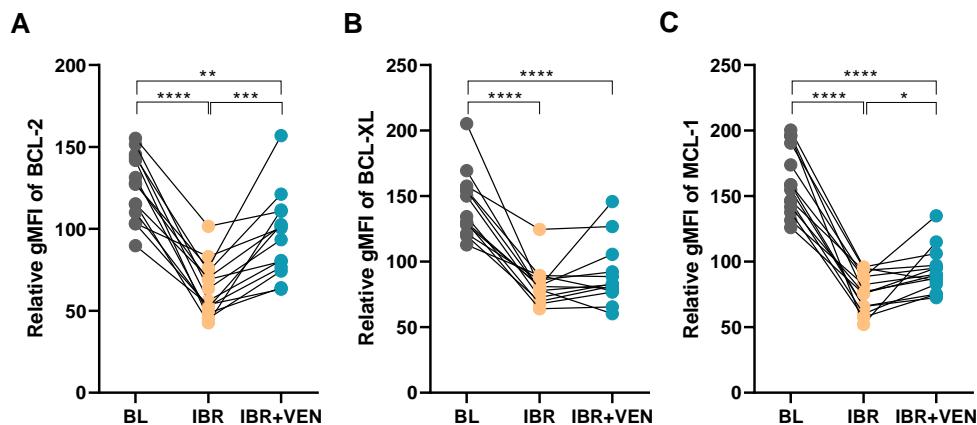


Supplemental Figure 5. Immunological detection of CD5 (A) and CXCR4 (B) in total CLL population before and after CpG stimulation (1μg/ml ODN2006)(N=4). Paired t-test was used for statistical analyses.



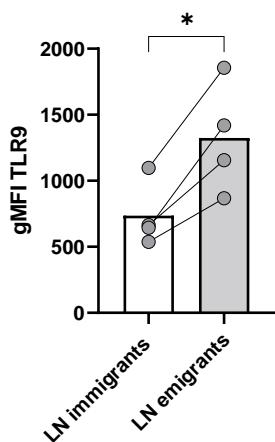
Supplemental Figure 6. Shorter *in vitro* venetoclax treatment does not affect venetoclax sensitivity after *in vivo* ibrutinib treatment

Ex vivo patients CLL cells obtained at baseline (BL) and after two months ibrutinib treatment (IBR) were treated with venetoclax *in vitro* for A) 1 hour (N=4), B) 2 hours (N=4) or C) 4 hours (N=6). Viability data was measured by flow cytometry using DiOC6/TO-PRO-3 staining.



Supplemental Figure 7. Bcl-2 family members after single ibrutinib treatment and combined venetoclax and ibrutinib treatment

(A-C) Immunological detection of Bcl-2 family members in LN emigrants before, after single ibrutinib treatment and after combined treatment with venetoclax and ibrutinib (N=14). GMFIs were normalized by setting the baseline LN immigrant population at 100%, and by subsequently plotting only the emigrant population. TWO-Way ANOVA test was used for statistical analyses.



Supplemental Figure 8. Immunological detection of TLR9 in LN emigrants (CD5highCXCR4dim) and LN immigrants (CXCR4highCD5dim)(N=4). Paired t-test was used for statistical analyses.